RAPID PLANT TISSUE TESTS AS A MEANS OF TESTING FOR MINERAL DEFICIENCIES IN PLANTS

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# RAPID PLANT TISSUE TESTS AS A MEANS OF TESTING FOR MINERAL DEFICIENCIES IN PLANTS

By

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To my wife

whose personality is undoubtedly

reflected in these pages.

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#### INTRODUCTION

Quantitative plant tissue analyses made in the laboratory have been used for many years as a means of studying the mineral requirements of plants. More recently an attempt has been made to place in the hands of the commercial grower a relatively simple method for the determination of mineral deficiencies in plants. These are the quick tests that have been devised for both plant tissue and for soil. The Purdue University kit was developed primarily for the testing of plant tissue, but, because the tests were adapted from the quick tests for soils, the kit can also be used in the testing of soil.

In the hands of the research workers a quick test kit of this type can be particularly valuable. As a general rule it takes eight to ten years of research work before general fertilizer recommendations can be made for a particular soil type and crop. This time could be reduced by at least half through the proper use of soil and plant tissue quick tests.

The test plot technique of fertilizer research is expensive, time consuming, and requires large areas of land. With the intelligent use of the quick tests and a small plot of land for each crop, results could be obtained in three to five years.

Some of the questions relative to these quick tests are:

- (1) Are they simple and rapid for the average person,
- (2) Are the results accurate and easily interpreted, and
- (3) Are the tests applicable to all crops?

These questions are of a practical and fundamental nature and must be answered before the value of a test kit of this type can be ascertained.

### REVIEW OF LITERATURE

Reports of work done relative to rapid chemical tests of plant tissue are rather limited and most of the work reported has been done since 1940. In 1945 Thomas (19) summed up the work of diagnosing mineral requirements of a plant by means of leaf analyses. He recognized quick tests at that time but most of his report was devoted to laboratory plant tissue analyses. According to Scarseth (16) the two methods are not comparable. Laboratory analysis measures the total quantity of an element in the tissue, both the quantity that has combined organically, as well as the inorganic reserves present. The quick tests, on the other hand, measure only the inorganic supply of an element or compound present in the tissue.

The earliest work was published by Carolus (2) in 1927. He sampled both field and greenhouse tomatoes that were well supplied with minerals and ran laboratory analyses for the inorganic mineral elements. These data were intended for use in determining the value of the quick tests.

Emmert (6) found that plant tissue tests showed the response of plants to nutrient treatments whether growing in soilless culture, in greenhouse soil, or in the field. In fact, he reported that plant tissue tests seemed to be a better measure of the nitrogen and phosphorous the plant was able to absorb than were the soil tests.

Scarseth (16) states that tissue tests indicate a nutrient deficiency before the leaves show starvation symptoms. Ulrich (22) also reported that the leaf blade of a tomato plant showed incipient potash deficiency before the petiole. Thus both of these workers indicate that when the plant tissue tests are used it is not necessary to wait for visual deficiency symptoms to appear before taking corrective measures.

Sampling techniques have been found to vary with different investigators.

Carolus (2) states that samples should be collected as soon as possible after the plant shows signs of deficiency because after a plant that indicates signs of some nutritional disturbance has been allowed to remain in the field for some time, a stage of premature physiological maturity develops which results in the formation of compounds in the plant that are very likely to cause off-color solutions in the various tests. This may be true, but as indicated above the visual deficiency stage need never be reached. Carolus (2) also states that samples should be taken from the lower half of the stems of long stemmed plants and from the lower petioles of leafy plants each in the stage of vigorous growth. This technique has been modified somewhat by later workers. Krantz (13) and his coworkers developed sampling procedures for several different crops. They found that the nitrates in corn decreased as the samples were taken closer to the tassel. In cowpeas the reverse was true. The nitrates increased as the samples were taken nearer the growing point. Thornton (20) and his co-workers are generally in accord with Krantz as to sampling techniques but differ in some cases on specific crops. For example, Krantz (13) selects the leaf blade of young corn plants and also states that these are satisfactory on older plants as well as stem tissue. Thornton (20) uses the base of the main stalk for nitrogen, the tip of the stem or below the tassel for phosphorous, and the base of a leaf at a node where an ear is produced for potassium. Thornton (20) also states that soybeans normally give a negative test for nitrate nitrogen while Krantz (13) says a soybean plant low in nitrates gives a positive test when the upper part of the plant is tested, and a negative test when the lower part is tested. This indicates a need for some standard procedure for the sampling of each crop to assure workers of results that will be truly comparable.

As a means of diagnosing mineral deficiencies in plants, Scarseth (16) points out the limitations of chemical soil tests. He says that plant roots

absorb elements from the soil slowly but continuously for several months, while in soil tests the solvents are in contact with the soil materials for only a few minutes. This is of the utmost importance where the dynamic, biotic soil complex is concerned. Also plant roots differ in feeding properties, for example, alfalfa and sweet clover will obtain more of the phosphates from an alkaline soil than corn or cotton. Soil tests, however, are usually designed for crops in general and not standardized as to a particular crop or soil. The pH of the extracting agent used in soil tests varies as compared to the pH of the root sap. Finally, plants absorb nutrients from subsoil as well as the topsoil and thus soil samples do not usually represent the entire root environment. When these limitations are taken into consideration, the rapid chemical soil tests can be invaluable in diagnosing the fertility situation in the soil.

As Krantz (13) states, the plant diagnostician must be well acquainted with the physiology of the plant if he is to make the most effective use of the plant tissue tests. Carolus (2), while working with tomatoes and spinach, found that a nitrogen deficiency in the plant resulted in a large accumulation of soluble phosphorous. Also where a phosphorous deficiency developed, large amounts of nitrate nitrogen accumulated. A potassium deficiency results in large accumulations of nitrates, magnesium, and calcium in the stems and petioles of the tomato and spinach plants. Finally, a magnesium deficiency results in low nitrates and high potassium in the stems and petioles of vegetable plants. Harrington (9) came to the same conclusion. He grew spinach in pots using Hoagland #1 solution (11) modified as necessary for the various nutrient levels required. He also found that the amount of minerals in the petioles varied with the age of the plant. In general nitrates decrease and phosphates increase as the plant ages, with little change in the potassium relationship.

Hoagland differs from Tottingham in the ability of a plant to absorb certain

nutrient elements from a soil that is low in those nutrient elements. Hoagland (11) states that a low potassium ion concentration in the soil solution does not necessarily mean that the plant itself is low in potassium. He showed that young barley plants were able to accumulate a high concentration of potassium in their roots even though the solution in which they were growing was very low in potassium. Meanwhile, Tottingham (21) states that the concentrations of phosphorous and potassium in the plant sap follow closely the relative supplies of these elements in the soil. When the soil is lower in minerals than the plant, minerals will pass out of the plant and into the soil through the roots.

Some investigations have been made for the correction of mineral deficiencies by the use of mineral sprays. Dickey (4) and his co-workers corrected a copper deficiency in tung trees by the use of an 8-8-100 copper lime spray. Tottingham (21) states that spraying with an iron sulfate solution will correct iron chlorosis. It is also well known that a zinc chlorosis condition of pecan trees known as pecan rosette can be corrected by spraying the foliage with a zinc sulfate solution. Lott (14) corrected a magnesium deficiency in Muscadine grapes by the use of both sprays and trunk injections of soluble magnesium. Along this line, but using a major element (nitrogen) instead of one of the minor elements, Fisher, Boynton, and Skodvin (7) sprayed apple trees with a urea solution and secured a significant increase in yield as compared to unsprayed check trees.

### METHOD AND PROCEDURE

Tomato plants and peach seedlings were selected as annual and perennial plants to check the results of the Purdue tissue test kit. Both of these are leading horticultural crops, and in addition they provide abundant foliage for testing material. They were grown in nutrient solutions with controlled mineral element levels of nitrogen, phosphorous, and potassium.

Field tests were made at the Vegetable Research Station at Bixby with plant material from the fall and spring crops of spinach. A small plot was marked off in field No. 4 for fertilizer treatment as indicated by the tissue test kit. Field No. 4 is one of four 10-acre rotation plots. It receives no fertilizer, and two cash vegetable crops are grown on it each year.

The winter tomato crop in the college greenhouse was tested and fertilized as indicated by the tissue test kit. The number and kind of fertilizer applications made were then compared to previous years' program of standard fertilizing procedures.

Applications of nitrogen fertilizers applied as sprays in order to get a more rapid absorption into the plant were also tested. This method has proven practical in the use of zinc for zinc deficiency in pecan trees, copper for copper deficiency in tung trees, and iron for clearing up iron chlorosis of shrubs and trees.

Collards and spinach were grown in flats and provided with various combinations of minerals. Mineral deficiencies, as indicated by tests, were corrected by the application of the deficient mineral in solution and tests were made again to determine how soon the deficient mineral was absorbed into the plant in ample quantities for growth. This was done to determine whether the test kit was consistent in its results on herbaceous annuals.

### A. The Purdue Tissue and Soil Test Kit

A description of the Purdue test kit and the chemistry of the tests seems desirable in order that the work be better understood.

The chemicals, glassware, and other supplies necessary for the soil and tissue tests are completely assembled in a convenient metal box. Brief instructions for making the tests are fixed to the under side of the lid so that when the box is open they are in a convenient position to be easily read.

The reagents for soil acidity consists of three indicators: No. 1 (brom thymol blue) to test for a pH range of 5.8 to 7.5; No. 2 (brom cresol green) to test for a pH range of 3.8 to 5.5; and Special Indicator (chlorophenol red) to test for a pH range of 4.8 to 6.2. The special indicator is for use when the soil acidity is near the lower limits of indicator No. 1 or upper limits of indicator No. 2.

The test for nitrates is used only when testing for nitrates in plant tissue. The reagent is made up by dissolving one gram of diphenylamine in 100 cc's of concentrated sulfuric acid.

The phosphate test can be used for tissues or soils. The extracting reagent for this test is ammonium molybdate dissolved in hydrochloric acid and distilled water. Either stannous chloride or stannous oxalate is used as the second reagent for developing the blue color.

The potash test can also be used for tissue or soil. The extracting reagent is sodium cobalti-nitrite dissolved in distilled water with acetic acid added. Reagent No. 2 is anhydrous isopropyl alcohol for use with the soil extract. Reagent No. 3 is 95% ethyl alcohol for use with the tissue extract. Charts are provided to make color and turbidity comparisons in order to evaluate the results of each test quickly.

The chemistry of the tests will give a better understanding of what is taking place and alleviate any doubt as to the practicability of the tests.

The nitrate test is adapted from a simple reaction of organic chemistry.

The phenyl groups are easily nitrated and the concentrated sulfuric acid merely removes the water from the reaction. The nitrated amine turns blue, and the intensity of the blue color depends on the amount of nitrates present.

The phosphates are extracted from the soil or plant tissue with an acid to keep the phosphate soluble. In the extracting process the ammonium molybdate picks up the phosphate forming a complex molecule of ammonium phosphomolybdate. The stannous chloride or oxalate is then added and acts as a reducing agent, reducing the molybdate ( $M_0O_4$ ) to molybdite ( $M_0O_3$ ). It is the changing of this part of the ammonium phospho-molybdate molecule that gives the solution its blue color. This is considered a very sensitive test, and the intensity of the color indicates the amount of phosphorous present.

In the potassium test another complex molecule is formed. Sodium cobaltinitrite combines with the potassium of the soil or plant to form the complex disodium potassium cobalti-nitrite  $[Na_2KCo(NO_2)_6]$  or sodium dipotassium cobaltinitrite  $[NaK_2Co(NO_2)_6]$ . This molecule, as such, is still highly soluble until the alcohol is added. Then, according to the theory of solubility, the molecule separates from the solution as a solid. Results in this test are determined by the turbidity of the solution and not the color.

#### B. Sampling

Sampling methods were kept as uniform as possible although some variance crept in of necessity. Tomatoes were sampled in the morning before eleven o'clock. When sampling the winter tomato plants in the college greenhouse, the plants selected were those that were most representative of the group.

The tomato plants were grown in a 30' x 100' house. The plants were spaced

in rows two feet apart and 21 inches in the row. The rows ran the length of the house. There were seven rows on each side of a center aisle three feet wide. The width of the house was also bisected by an aisle three feet wide. If the rows are counted from the center aisle to the outside, rows 1, 3, 4, and 7 were buffer rows set up to check on the effect of fertilizer applications indicated by the tests. This allowed two identical treatments, one on each side of the house. The plants selected for sampling were selected from the test rows between the buffer rows. The most recently matured leaves, one from each of six plants, were taken and stripped of their leaflets. A sharp knife was used to cut thin sections starting from the base of the petioles and proceeding upward until sufficient tissue had been cut for testing. Since each sample of leaf petioles was composited when cut, it was necessary to cut only a little more sample than was needed for the test. Two or three tablespoonfuls of sliced material is more than enough.

Sampling of the tomato plants and peach seedlings grown in pots under controlled conditions to check the results of the test kit was slightly different. There were only three of each plant in each plot which made it necessary that samples be taken from all three plants. The peach seedlings did not produce as much foliage as was expected thus making it necessary to remove the leaf blade and use part of the midrib. These plants were also sampled in the morning before eleven c'clock.

Spinach sampling at the Vegetable Research Station was done between eleven o'clock in the morning and four o'clock in the afternoon. Ten to twelve plants were selected at random, as nearly representative of the entire field as possible. One of the most recently matured leaves was picked from each plant. The petiole was saved for testing and the leaf blade was discarded. Because of the habit of growth of the plant the petioles were often contaminated by soil particles.

These were cleaned by blowing off the loose soil particles and wiping those that adhered with a clean cloth.

Soil sampling varied with the location. Samples were taken from the one gallon crocks at two or three places in each crock using care to avoid getting roots with the sample. A tablespoon measure was used in taking the samples so that the composited sample from the three crocks in each plot amounted to six to nine tablespoonfuls of soil. An eight inch U channel probe was used in the tomato greenhouse for procuring soil samples. Soil was obtained to a depth of about six inches. Three probes were made at random in each of the two sections of the house receiving different fertilizer treatments. These also were composited and tested. At the Vegetable Research Station a thin soil slice was taken to a depth of six inches in at least six different locations selected at random. These were composited and tested.

### C. Treatments

1. Tomato plants and peach seedlings in the greenhouse with controlled nutrient level of the growing medium.

The growing medium used for the plants was washed river sand, screened through ordinary window screen to remove the coarser particles. This was done in order to improve the moisture-holding property of the sand. Soil tests were made of the sand to insure that it was low enough in minerals so as not to interfere with the desired mineral element levels. These tests were made with the Simplex soil test kit and showed only traces of nitrogen, phosphorous, and potassium with a pH of 7.0. The sand was sterilized to prevent disease and to destroy seeds that might interfere with the tests.

The tomato plants and peach seedlings were transplanted into the sand in one gallon crocks which were provided with drainage holes. The

peach seedlings were dug from the field and transported wrapped in wet burlap. The roots were washed clean in water, pruned along with the tops and immediately planted in the crocks. The tomato plants were of the Master Marglobe variety and were those which remained from the greenhouse planting. They were about 18 inches tall and rather potbound when transplanted. The soil was washed from the roots as completely as possible, and the plants were immediately planted in the crocks. In order to prevent contamination of the roots the crocks were placed in 3' x 6' tin pans coated with emulsified asphalt and provided with adequate drainage. Ten pans containing three peach seedlings and three tomato plants each were set up. The six plants in each pan were treated to maintain the same mineral element level as indicated:

Treatment of each plot.

- (1) check
- (2) minus nitrogen
- (3) minus phosphorous
- (4) minus potassium
- (5) low nitrogen
- (6) low phosphorous
- (7) low potassium
- (8) high nitrogen
- (9) high phosphorous
- (10) high potassium

Hoagland and Arnon (11) solution No. 1 was used as the basis for the various nutrient solutions. The check plot used the full strength solution. The minus plots had the solutions modified to remove only the one element as indicated. The solutions for the low plots were

modified to decrease concentration by one-half that of full strength for each plot as indicated. The solutions for the high plots had the concentration of the elements doubled as compared to standard solution.

To start the plants, alternate applications of 200 cc's of the various solutions and water were made every other day. This was gradually increased for the tomato plants as they grew larger. The peach seedlings, on the other hand, began to show signs of too much moisture and it became necessary to reduce the feedings to once a week, alternating with 100 cc's of solution and distilled water each week.

Each tomato plant was trained to four stems which were sufficient to furnish the material required for the tests.

The peach seedlings were pruned severely to force new vigorous growth. It was thought that transplanting in late September might force them into a rest period. However, such was not the case; they suffered a leaf drop and then resumed vigorous growth.

2. Tomatoes in the college greenhouse grown as a commercial crop.

The history of the greenhouse soil dates back fourteen years. It was originally a Kirkland series, very fine sandy loam, eighteen inch clay hardpan. Two inches of sand were added the first year to loosen the soil and allow for better drainage. In addition, that year and each year thereafter, strawy manure was added to the soil at the rate of forty tons per acre. Commercial fertilizers are added each year just before the young tomato plants are transplanted.

	Fertilizor	Rate of Application	
Superphosphate 1000 per acre	Superphosphate	1000 per acre	
KCl or $K_2SO_4$ 750 per acre	KCl or K <sub>2</sub> SO <sub>4</sub>	750 per acre	

 Table 1. Commercial Fertilizer Application Made Each Year

 Before Transplanting Young Tomato Plants

Sulfur and ferrous sulfate were added to the soil in 1940 to lower the pH. The soil has shown a lack of manganese as evidenced from visual foliage deficiency symptoms. Seven applications of manganese sulfate have been necessary since 1937 to correct this deficiency.

As the 1948-49 crop developed it was fertilized as indicated by tissue tests.

3. Spinach grown as a field crop at the Vegetable Research Station.

The history of the soil on the Vegetable Research Station is brought in here to give a more complete picture of the situation there. The soil is of the Yahola series, a very fine sandy loam bottom-land that is high in natural fertility. The crop rotation block of 40 acres (Chart 1) is laid out in four ten-acre fields. Each field receives a different treatment. Field No. 4 has been used as a check plot for the other three fields. Two cash crops a year are grown on it without fertilizers or soil-building crops. Field No. 2 also has two cash crops grown but with fertilizer added to each crop and soil-building crops grown and turned under whenever possible. Those treatments and rotations have been carried on for the last five years.

Plots A and B (Chart 1) are areas used for vegetable variety and yield tests.

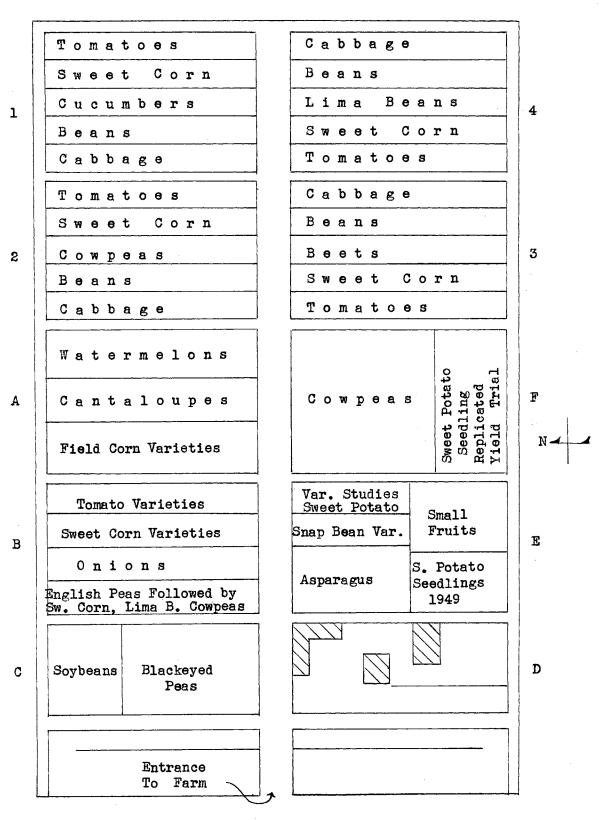


Chart 1. Diagram of the Vegetable Research Station at Bixby Showing the 1949 Spring Crops. Fields No. 1 - 4 are the Rotation Fields.

When deficiencies were indicated in the rotation plots by the quick tests, a small area was blocked off in the plot showing a deficiency for fertilizer treatment as indicated by the tests. In contrast to this, the entire planting of spinach in plot B was fertilized as indicated by the tests.

4. Using hitrogen sprays on spinach to test the absorptive power of spinach foliage for nitrogen.

The test was made three different times. Each time spinach seed was planted in four flats. Two of the flats contained sterilized river sand and two, sterilized soil. One flat each of sand and soil was tested while the others were used as checks. The plants were grown with nutrient solution until large enough to furnish sufficient tissue for testing. Then the plants were given only water until tissue tests indicated a minus nitrogen condition in the plants. At this time a Uramon solution, 2 percent nitrogen by weight, was applied to the leaves with a small brush to provent any solution from reaching the soil which would be the case if the plants were actually sprayed. Tissue tests were made hourly to detect any possible nitrogen absorption and to determine the length of time required for nitrogen absorption by the plant.

In the second test, Uramon and ammonium nitrate solutions, 2 percent nitrogen by weight, were both used in separate applications. In the third test, Uramon and ammonium nitrate solutions at a concentration of 2 percent Uramon and ammonium nitrate by weight were used because of excessive leaf burn caused by the higher concentration.

The flats used in the second and third tests were supported above the soil in the bench thus avoiding all chance of contamination where

the flats were in contact with the soil.

5. Use of the test kit on spinach and collards, (a greenhouse test).

Sixteen flats of sterilized, unscreened river sand were prepared and half of them seeded with spinach and the other half with collards. The flats were placed in large tin pans, four to a pan to keep the roots out of contact with the soil beneath the flats. The tin pans had sufficient drainage so that nutrient solutions would not accumulate around the flats. The plants were provided with complete nutrient solution until large enough for sampling, after which they were provided with various combinations of elements as indicated:

(1)	minus	(5)	N P
(2)	N	(6)	NK
(3)	P	(7)	PK
(4)	ĸ	(8)	NPK

The magnesium, calcium, and minor elements were included in each solution as these solutions were modifications of Hoagland and Arnon solution No. 1 (11).

Tests were made to be certain that the minerals to be checked were high in all plants before applying the deficient solutions. As soon as a deficiency was noted, it was corrected and the time noted when the tests showed that the plants in that particular flat were no longer deficient. This treatment was carried out for all of the flats of spinach and collards.

#### PRESENTATION OF DATA

1. Tomato plants and peach seedlings in controlled nutrient solutions.

Because the tissue test kit was to be used on plants with unknown nutrient reserves this part of the test was set up to see what the tests would show when the level of mineral elements in the plant was known.

The application of nutrient solutions to the plants was started October 27, 1948. The following day tissue tests were made on the tomato plants in each plot to determine their nutrient level at the start of the experiment. (Table 3) There wasn't enough tissue for tests on peaches at this time so tissue tests were carried out only on the tomato plants.

The pH of the soil in each plot (Table 2) and the nutrient solutions (Table 4) were also tested at this time. The soil pH ranged from 7.0 to 7.4 while the pH of the nutrient solution ranged from 5.5 to 6.4. It was decided that the soil pH would shortly come down to the desired level due to the lower pH of the nutrient solution. Seventeen days later the soil pH ranged from 6.2 to 6.6.

In the minus nitrogen plot it was interesting to note that while one of the tomato plants showed definite signs of nitrogen deficiency, the other two plants were of the same size and color as the plants in the check plot. Nitrate soil tests were made with the Simplex soil test kit, and only a trace of nitrogen could be detected in the soil in which all three of these plants were growing. Tissue tests showed no nitrates in the plant showing deficient signs and only traces of nitrates in the two good plants. This would seem to indicate that the nitrate test can detect a nitrogen deficiency in the plant before it becomes visible in the appearance of the plant.

When the plants which were given deficient solutions developed typical

		Plot Treatments											
Date	Test	Minus N	Low N	High N	Minus P	Low P	High P	Minus K	Low K	High K	Check		
Oct. 28,48	pH	7.4	7.4	7.2	7.4	7.0	7.4	7.4	7.4	7.2	6.6		
Nov. 1,48	DH	7.0	7.2	7.0	7.0	7.4	7.0	7.4	7.4	7.4	7.4		
Nov. 1,48	P	N	VII	Ħ	L	M	R	H	VH	H	Ħ		
Nov. 1,48	K	闼	I	L	Ρζ	N	н	VI.	L	И	M		
Nov. 18,48	PH	6.6	6.6	6.6	6.6	6.2	6.2	6.6	6.6	6.6	6.6		
Dec. 9,48	PH	6.8	6.8	6.6	6.6	6.6	6.8	6.8	6.6	6,6	6.7		

## Table 2. Soil Tests Made on Each Plot to Check Progress of pE Change in the Soil.\*

Table 3. Tissue Tests Made at the Beginning of the Test.

Date	Test	Minus N	r V	High N	Minus P	Low P	High P	Minus K	Low K	High K	Check
Oct. 28,48	N	н	VН	VH	VH	VH	VH	VH	VH	М	Н
Oct. 28,48	P	VH	VH	M	M	H	H	Di	L	Ħ	ΨН
Oct. 28,48	K	н	VH	VII	VH	VH	H	νн	н	H	Ħ
a a fair a fair a fair ann an tha											

Table 4. pH of Nutrient Solutions.

		1				10.40° - 003 400 - 10- 00- 00-	anteren an	Contraction of the local date	C. D. Martin Cont. and Cont. Spinster,	Party dates Suffrage Vieland
Date	Minus	Low	High	Minus	Low	High	Minus	LOF	High	Check
	N	N	N	P	P	Р	K	K	ĸ	
Oct. 28,48	6.4	5.8	5.7	7.4	5.7	5.5	5.8	5.6	5.8	5.8

\*Interpretation of nutrient levels:

	minus	3	11	mediu	un
VL	very	low	H	high	
L	low		VE	very	high

deficiency symptoms, tissue and soil tests were made of every plot. (Table 5) The results of the tests for each plot are given in two ways: (a) the results of the tests are described for the element that is varied, and (b) the levels of the two elements present in normal amounts are given. This was done so that results of later tests could be compared when necessary and as an aid for the determination of results by other workers.

Sufficient foliage for the scheduled tests on peach seedlings did not develop. However, the tests (Table 6) made indicated that the nutrient conditions within the peach leaves could not be as readily measured as in the tomato plants. It has been found, that with certain modifications,<sup>1</sup> these tests can be used on peach trees.

The tissue tests showed a very close relationship between the treatment, the results of the test, and the color charts. The soil tests for phosphorous (Table 7) were badly out of line. Further tests for phosphorous were carried out (Table 8). It was found that by adding phosphorous reagent No. 2 ( $Sn_2Cl_2$ ) until the blue color could no longer be intonsified, the results fell in line with the treatments.

The nitrogen test was modified slightly in an effort to get a greater degree of accuracy. The procedure carried out amounted to putting several pieces of cut tissue in a vial and adding enough reagent to cover. The vial was then rotated to get some reagent on the sides of the vial. A color standard was selected based on the results of the nitrate tests in Table 5 which is as follows:

minus nitrogen. . . . . . no blue color low nitrogen . . . . . blue but doesn't coat sides of vial medium nitrogen . . . . . coats sides of vial a medium blue high nitrogen . . . . . . coats sides of vial a dark blue

1 Correspondence with Purdue University horticulture staff.

Table 5. Results of Tissue Tests on Tomatoes with Controlled Nutrient Levels. A Description of the Results is Given for the Element that is Variable in Each Plot.

Plot	Test	December 22, 1948	January 12, 1949
Minus N	N K P	no blue color very high low	no blue color very high medium
Low N	N	coats sides of vial a medium blue	pale blue color
	K P	very high very high	very high high
High N	N	coats sides of vial a dark blue	coats sides of vial a very dark blue
	K P	very high medium	very high high
Minus P	Р	greenish colored liquid	blue-green liquid
	N K	medium very high	high very high
Low P	P	a blue-green color	darker blue-green than minus P
	N K	medium very high	high very high
High P	Р	dark blue approaching navy blue	dark blue approaching navy blue
	N K	high Very high	very high very high
Minus K	K	orange color, slightly cloudy	no precipitate
	N P	medium low	high high
Low K	K	almost opaque, yellow- orange color	opaque, but not as yellow as high K
	N P	high very high	high very high
High K	ĸ	opaque ppt., color yellow	opaque ppt., very yellow as
	N P	as in chart very high high to very high	in chart very high very high
Check	N	coats sides of vial	coats sides of vial dark
	K	dark blue opaque ppt., color yellow	blue opaque ppt.
	P P	as in chart medium blue color	medium blue color

Table	6.	Resul	ts of	Tiss	uo	Tests	on	Peach	Seedlings	
		with C	ontro	lled	Nut	rient	Le	vels.		

	Plot Treatments									n na far neft ær far fær i sjører som stør over i sjører støre sjøre som en en efter som en efter som en efter				
Date	lest	Minus N	Low N	High N	Minus P	Low P	High P	Minus K	Low K	ĸ	Check			
Jan. 12,49	N	minus		minus						minus				
	р				med.		ned.			ned.				
	K							med.		med.				

Table 7. Results of Soil Tests on Soil of Tomato Plants and Peach Seedlings in Each Plot.

an mangang sa ang sa		Plot Treatments											
Date	Test	Minus N	Low N	High N	Minus P	Lou P	High P	Minus K	Low K	High K	Check		
Jan. 5,49	pH	5.8	6.6	5.8	6.6	6.0	6.0	6.0	6.6	6.6	6.6		
	P	H	H	H	H	H	VH	Ħ	н	H	H		
	K	lì	M	E	H	36	н		L	AH	24		

Table 8. Recheck of the Soil Phosphorous Tests.

	Jan. 12	Jan. 13	Jan. 14
Minus P	medium	medium	medium
Low P	m⊖ <b>diu</b> m	modium	medium
Check	medium	very high	very high
High P	medium	very high	very high

These tests provided a good basis or standard for tests to be made later on plants of unknown levels of reserve minerals. They also indicated that it was possible to determine when an herbaceous annual plant was becoming deficient in a major element (N, P, or K) before the plant actually gave any visible indication of this deficiency. This is very important if plants are to mature quickly and attain their full growth and maximum yield.

To make the test complete, at this stage a complete nutrient solution was applied to the deficient tomato plants. The plants were tested each day to determine when they were able to absorb more than enough of the deficient minerals to satisfy their needs. The time was also noted when the plants showed visible signs of recovery and was compared to the time the tissue tests indicated recovery.

As was expected the tests gave positive results before the plant showed definite signs of the resumption of normal growth (Tables 9 & 10). Within three days tests for nitrogen, phosphorous, and potassium showed traces of these elements. Nitrogen on the nitrogen deficient plant and potassium on the potassium deficient plant tested very high within seven days and phosphorous within nine days. The potassium deficient plants showed increased vigor of growth eight days later, the nitrogen and phosphorous deficient plants nine days later. The tests indicated sufficient mineral in the tissue for recovery at least five days before evidence could be detected visibly in the plant.

Table 9. Results of Tissue Tests Made of the Deficient Tomato Plants After 0915, February 8, 1949 When They Were Given a Complete Nutrient Solution to Show When Recovery was Indicated.

Date Time Test Plant Tested Results			นการสารและสารแกรงการสารแรงการสารแรงการสารแสดงการสารและสารและสารและสารและสารและสารและสารและสารและสารและสารและส สารแสดงการสารและสารและสารและสารและสารแสดงการสารแสดงการสารแสดงการสารแสดงการสารแสดงการสารแสดงการสารแสดงการสารแสดง 1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.		
Jate	TTWG	rest	rlant lested	Results	
Feb. 8	0915	N	minus N	very pale blue	
	1430	И	minus N	no blue color	
Feb. 9	1000	N	minus N	no blue color	
Feb. 10	0900	N	minus N	no blue color	
Feb. 11	1030	N	minus N	pale blue (3 tests)	
		P	minus P	bluish green	
		K	minus K	slightly cloudy	
Feb. 12	0900	N	minus N	pale to medium blue (3 tests)	
Feb. 13	1100	м	minus N	medium to dark blue (3 tests)	
Feb. 15	<b>09</b> 00	N	minus N	dark blue (2 tests)	
		P	minus P	greenish blue	
		ĸ	minus K	heavy ppt., opaque	
Feb. 16	1000	N	minus N	dark blue (2 tests)	
Feb. 17		N	minus N	dark blue	
		P	minus P	dark blue	
Feb. 19		N	minus N	dark blue	
		Р	minus P	dark blue	
		ĸ	minus K	heavy ppt., opaque	
C-100 - 10-10-10-10-10-10-10-10-10-10-10-10-10-1		1	a a balance and the state of the second s		

Date Plants Observed					
	Laura Opsolved				
Feb. 16	minus K plot showing visible signs of recovery				
Feb. 17	all plants show signs of renewed vigorous growth				

Table 10. Dates upon which Deficient Tomato Plants Showed Initial Visible Signs of Recovery.

2. Tomatoes grown in the college greenhouse as a commercial crop.

Tissue tests of the tomato plants growing in the college greenhouse were made at weekly intervals during the 1948-49 season. Soil tests were made often enough to serve as a check on the tissue readings. The purpose of this part of the experiment was to determine if the nutrient level of soils could be maintained at a desirable level by fertilizing as indicated by tissue tests alone. Table 11 shows the results of both the tissue and soil tests. Results as indicated in the table point to a close correlation between the two tests.

The early deficiency of phosphorous indicated by both the soil and tissue tests is somewhat questionable because of the fertilization treatment of the soil before the tomato plants were set and because of the inherent nature of phosphorous in not being readily leached from the soil. A phosphorous deficiency by October 16, 1949 was considered quite unlikely. It was brought out earlier that in the test for phosphorous, unless enough reducing agent is used, the maximum intensity of color is not reached and a low reading is likely to result.

Two phosphorous fertilizer treatments were made on the basis of the tests indicating a deficiency of phosphorous. Superphosphate was applied to the test rows at the rate of 200 pounds per acre and ammonium phosphate was applied to the check rows at the rate of 200 pounds per acre.

By April 2 the tissue tests indicated that a general nitrogen deficiency



Figure 1. Tomato Plants in the College Greenhouse Shortly After Being Transplanted.



Figure 2. Tomato Plants in the College Greenhouse About 30 Days After Transplanting, Showing Method of Tying and Training. existed. Consequently ammonium nitrate at the rate of 200 pounds per acre was applied. This application was made throughout the greenhouse instead of in plots because the practice of fertilizing the check rows as was done in previous years was not kept up. The fertilizer was not watered in because of unfavorable weather<sup>2</sup> until April 28 and consequently the tests did not indicate that the plants had recovered until April 29. Other than this, manganese sulfate was added to the soil early in December to correct a manganese deficiency that had been gradually becoming more severe.

Previous years' fertilizer practice stands out in sharp contrast to that of the current season. In 1948 the fertilizer applications were made as follows:

(1)	$(\mathrm{NH}_4)_2\mathrm{SO}_4$	200# per acre	after 4th clustor set fruit
(2)	$(NH_4)_2SO_4$	200# per acre	October 25
(3)	$(\mathbb{NH}_4)_2$ SO $_4$	200∦ per acre	November 29
(4)	$(\mathrm{NH}_4)_2\mathrm{SO}_4$	200# per acre	December 15
(5)	$(NH_4)_2SO_4$	200 $\#$ per acre	January 4
(6)	K <sub>2</sub> SO <sub>4</sub>	200 $\#$ per acre	Janua <b>r</b> y 4
(7)	$(\mathrm{MH}_4)_{\mathrm{S}}\mathrm{SO}_4$	$200^{\prime\prime}_{ll}$ per acre	April 5

Yearly yield figures cannot be compared because those for 1948-49 are not available at this writing. Results were judged mainly by the appearance of the plants and color of foliage.

Plants showed no signs of a nitrogen, phosphorous, or potassium deficiency throughout the growing season. Except for a manganese deficiency which was corrected, the color of the foliage remained a healthy green during the entire season.

The foregoing indicates that tissue tests may be used successfully in determining and remedying nitrogen, phosphorous, or potassium deficiencies in greenhouse tomatoes before plants show visible evidences of these deficiencies.

<sup>&</sup>lt;sup>2</sup> Overhead watering to dissolve the fertilizer is only done when it is evident that the sun will be visible the entire day in order to dry the foliage and prevent the spread of fungue diseases.

Date	Plants Tested	$\frac{\mathbf{Tissue}}{N} \frac{\mathbf{Test}}{P} \frac{\mathbf{Test}}{K}$	ts Soil Tests pH P K
Oct. 16,48	healthy leaves yellowed leaves (Mn deficient)	HMVI HHVI	
Nov. 6,48	N.W. quarter N.E. quarter	VH L VI VH L VI	1
Nov. 13, 48	N.W. quarter N.E. quarter	VH L. VI VH L. VI	
Nov. 19,48	N.W. quarter N.E. quarter	VH L VI VH L VI	
Nov. 27,48	superphos treated anmophos treated	AH AH AH AH AH AH	
Dec. 4,48	superphos treated ammophos treated	AH AH AI AH AH AI	
Dec. 11,48	superphos treated ammophos treated	AH H AI AH H AI	
Dec. 18,48	superphos treated ammophos treated	AH H AI AH H AI	
Dec. 28,48	superphos treated anmophos treated	VH H VI VH H VI	
Jan. 3,49	superphos treated ammophos treated	AH AH AI AH AH AI	
Jan. 15,59	superphos treated ammophos treated	AH AH AH AH AH AH	
Jan. 24,49	superphos treated ammophos treated	AH AH AH AH AH AH	
Feb. 5,49	superphos treated amnophos treated	AH AH AH AH AH AH	
Feb. 12,49	superphos treated ammophos treated	AH AH AH AH AH AH	
Feb. 26,49	superphos treated ammophos treated	AH AH AH AH AH AH	1

## Table 11. Results of Tissue Tests and Soil Tests Obtained Throughout the Growing Season on Greenhouse Tomatoes Grown as a Commercial Crop.

# Table 11. (continued)

Date	Plants Tested	Tiss N	ue To P	oste <u>K</u>	Soil Tests pH P K
Mar. 5,49	superphos treated anmophos treated	VE VE	VH	VH VE	
Mar. 12,49	superphos treated ammophos treated	VH VH	VH VH	AH AH	5.2 VH VH 5.2 VH VH
Mar. 19,49	superphos treated ammophos treated	H VH	AH AH	NH HV	
	Recheck on nitrogen (2) VH, (3) M, (4		i samj	ples):	(1) VH,
Mar. 26,49	superphos treated annophos treated	H H	VH VH	VH HV	5.0 VH VH 5.0 VH VH
Apr. 2,49	superphos treated armophos treated	H	AH AH	VH VH	
Apr. 9,49	superphos treated amnophos treated	L L	VH VH	VII VII	
Apr. 16,49	superphos treated annophos treated	L L	NTI HA	NH HV	
Apr. 29,49	superphos treated annophos treated	II E	HV HV	VH VH	
May 7,49	superphos treated anmophos treated	AH Mi	VH	VH VH	
May 14,49	superphos treated ammophos treated	M M	VH VH	VH HV	

It must be borne in mind that there are many other factors that must be considered. Our experience was also concerned with wilt, manganese deficiency, and red spider which had to be recognized and the proper controls applied.

3. Spinach grown in the field at the Vegetable Research Station.

Field No. 4, the check field in the rotation block, would be expected to show signs of mineral deficiency before Field No. 2 because of the soil depleting nature of the cropping program practiced. Tests were started while the plants were young. Evidently the young plants were not suffering from competition for mineral elements (Table 12) because the tests indicated that all three minerals (nitrogen, potassium, and phosphorous) were high. The color of all of the fields of spinach was very good at this time, also indicating a sufficient supply of minerals evailable.

The November 10 tests indicated low phosphorous in both fields. This is probably another instance of the misuse of the phosphorous test as was brought out earlier. Subsequent tests showed that nitrogen, not phosphorous, was the deficient element.

> Chart 2. Field No. 4 showing where the spinach was cut and where the samples were taken.

(a)	cut	(b)	N N
to construct on the second	10/~10/10/10/10/10/10/10/10/10/10/10/10/10/1	n Perfektiver and the contract of	

Date	Test	FLOID	a #4	Field #2
مەربىلىقىنىڭ مەربىلىدىنىڭ تەربىيىتىنىڭ ئەربىيى ئەربىيىلىقىنىڭ ئەربىيىلىكى ئەربىيىكى ئەربىيىكى ئەربىلىكى ئەربىي ئىلىنىڭ ئەربىلىكى ئەر	ananiana, kitalan dan kitanan saina katakan saina da	unfertilized*	fertilized	an and a surface of the surface of t
Oct. 14,48	N	H		H
	P	VH		VH
	K	VH		VH
Nov. 10,43	N	VH		AH
	P	L		L
Dec. 16,48	N	-		VH
	P	<u>r</u>		H
	K	VH		HV H
Jan. 6,49	N	426		VH
	P	11		VH
	K	<b>A</b> H		HV
T 01 10	N		VEI	
Jan 24,49	7.0		ALT	
Mar. 12,49	N		н	WH
DICELS THE TO	20	M	1 IE	NT N
	ĸ	AH	VH	VE
	~	W sofees		
Mar. 24, 49	N	ΔT	VL	M
	P	VH	Ħ	VII
	K	VII	VH	VH
Nar. 31,49	N	<del>ال</del> ت	VH	AH
	P	Ħ	L	AFI
	K	<b>A</b> H	VH	HV
Apr. 5,49	N	4 <b>3</b>	VH	
	P	VH	VH	
	K	VH	VH	
egymen tadwe tidate o stratam, jaking tada (ever - vrzedaniania, dolika danada zakarzate do strata zakarzategi - statu e statu zakarzategi - statu zakarzategi - statu zakarzategi - st		የሚያለው, በጋን የሚመለት, ለመቀር የሚያስር መንስ መንስ የሚያስር ጊዜ የተሰጠ አለም የሚያስ መቆጠረው ላይ ማይሻር መሻር ለማምር ጊዜ የሆኑ የሚያ የሚያስር መስለ ይህ በመስለ የሚያስር የሚያ		

Table 12. Results of Tissue Tests of Spinach in the Field at the Vegetable Research Station at Bixby.

\*Tissue tests made before January 6,49 wore not localized in the field and were subject to variations in the field. By December 16 a strip of spinach through the center of Field No. 4 had been cut for market. Extreme variability of fertility within the field showed up at this time. Tissue tests of a sample taken from the "a" area indicated a lack of nitrogen as was expected by the yellow color of the plants. Tests of a sample taken from "b" area indicated a large amount of nitrogen available which was also apparent by the healthy green color of these plants.

An area 10 feet square was measured for treatment in the part of the field showing low nitrogen. Uramon was applied to this area at the rate of 70 pounds of nitrogen per acre. The object was to use the tissue tests to indicate which element was limiting the growth of the spinach and then show that it could be corrected by fertilizing on the basis of these tests.

Tests were made again on January 6 on the fertilized area of Field No. 4. It was found that no nitrogen had been taken up by the plants. This was explained by the fact that with the cold weather the soil bacteria were inactive and did not break the Uramon down from its organic composition to an inorganic form easily absorbed by the plants. In view of what had happened, an application of ammonium nitrate was made at the same rate of application as the Uramon to the same area.

On January 24, a nitrogen test of the area fertilized with ammonium nitrate in Field No. 4 was made by the farm superintendent. He described his results as indicative of very high nitrates in the plants.

As indicated by the early tests (Table 12) nitrates were low in Field No. 4, thus the results obtained by fertilizing with amnonium nitrate verify these tests. Tests made again on March 4 indicated low nitrates and were followed by another application of ammonium nitrate on this date at the rate of 70 pounds of nitrogen per acre. The last two tests before the spinach was ready for harvest indicated that a sufficient amount of nitrates was present for normal



Figure 3. Spinach in Field #4 Showing the Corrected Nitrogen Deficiency in a 100 Square Foot Plot.



Figure 4. Bird's Eye View of Spinach in Field #4 Comparing the Lateral Growth of the Plants in the Nitrogen Corrected Plot and the Remainder of the Field. healthy growth.

The phosphorous tests made on the 100 square foot plot indicated that the phosphorous supply varied somewhat after January 6 in accordance with the relationships brought out by Carolus (2). After January 6 (Table 12) the phosphorous tests indicated an increase of phosphorous with a decrease of nitrogen and a decrease of phosphorous with an increase of nitrogen.

During this time the unfertilized area of Field No. 4 consistently gave negative tests for nitrogen and the plants remained yellow and small. In contrast to Field No. 4, Field No. 2 had an adequate supply of minerals which was indicated by the tissue tests as well as the color and growth of the plants.

One other factor may be brought out as a result of a comparison of soil and tissue tests. Soil tests didn't show dangerously low amounts of phosphorous in the soil. However, the tissue tests on March 31 indicated a low reserve of phosphorous in the plants. Thus it seems safe to conclude that when all other elements are abundant the level of phosphorous, as indicated by soil tests, is inadequate. In addition, the low test for phosphorous was obtained one week after an abundant application of nitrogen was made. This is also in accord with the work of Carolus (2).

4. Nitrogen sprays on spinach to test absorption through the foliage.

When testing for nitrogen in the spinach plants the question arose as to how soon one might expect nitrogen to be absorbed in large enough quantities to be detected by tissue tests. It was anticipated that this would take place within a matter of hours. The first tests were made at hourly intervals after the foliage application of the Uramon solution. The spinach plants in all four plots were checked for nitrogen level before the spray application. The sand flats indicated minus nitrogen and the soil flats indicated medium nitrogen. The sand and soil flats were set up to contrast one against the other.

Date	Test	Field		Field #2
		unfertilized*	fertilized	
Oct. 14,48	pH	6.6		6.2
	P	M		H
	P K	VH		VH
Dec. 16,49	pH	5.6		5.6
	P K	Н		VH
	K	VH		VH
Mar. 12,49	pH	6.8	6.6	6.6
	P	Н	H	VH
	pH P K	H	ΥH	VH
Apr. 5,49	pH	6.6	6.6	
	P	VH	VH	
	K	H	VH	

Table 13. Results of Soil Tests of Spinach in the Field at the Vegetable Research Station at Bixby.

\*Soil tests made before March 12 were not localized in the field and were subject to the variations of the field.

Table 14. Nitrogen Tests Made of Spinach Sprayed with Uramon (2% nitrogen solution) at Hourly Intervals. Plants Sprayed at Eleven O'clock After Check Tests Were Made.

		Sand Flats		Soil Flats		
Date	Time	sprayed	check	sprayed	check	
1	1100(chk)		-	M	M	
	1200	-	-	VH	M	
	1300	-	-	VH	M	
	1400	-		VH	M	
Dec. 12,48 1100		varied fr high (5 t		VH	H	

Referring to Table 14, the spinach in the sand flats showed no increase of nitrogen in the tests made that day while the spinach in the soil flat showed an increase when compared to the check. The next day tests made on the spinach of the sand flat were very inconsistent, from minus nitrogen to high. In an effort to find a reason for these inconsistencies the flats were raised and it was found that the roots of some plants had penetrated to the bench soil. The conclusion drawn from this test was, that because all plants were treated but not all plants indicated an increase in nitrogen, they did not absorb enough nitrogen to be reflected by tissue tests.

The second and third tests were corrected by setting the flats of spinach on empty flats to prevent the roots from making contact with the bench soil. The tests were made daily because hourly tests showed no increase of nitrogen. The concentration of nitrogen was reduced from 2 percent nitrogen to 2 percent Uramon and ammonium nitrate for the last test because of severe leaf burn encountered with both Uramon and ammonium nitrate. One other refinement used in the last tests was the addition of a spreader, a commercial preparation called "Wondrop", to the Uramon solution to get a more uniform coverage of the foliage.

The results are shown in Tables 15 and 16. At no time was the nitrogen absorbed in large enough quantities to be detected by the tissue tests. This is not to be construed to mean that it is not a practical method for fertilizing spinach. Results obtained by other workers, mentioned earlier, refute this; however, it does indicate that tissue tests do not detect nitrogen absorption as Uramon and ammonium nitrate through the leaves to any degree.

5. The use of the test kit on spinach and collards. (a greenhouse test)

At the time the collards and spinach plants were considered large enough for testing, nitrogen tests indicated that the plants were low in this element. The plants were to be given different combinations of nitrogen, phosphorous,

Table 15. Nitrogen Tests Made of Spinach in Flats of Sand Sprayed with 2% Nitrogen Solutions of Uramon and NH4NO3. Plants Sprayed March 8, 1949 at 1530 O'clock.

Date Time Mar. 9,49 1020		Uramon sprayed check		NH4N0 sprayed	check
		(2 tests)		inconsistent (5 tests)	-
Mar. 10,49	1000		-		-
Mar. 12,49	1000	(leaf burn	- very bad)	-	-

Table 16. Nitrogen Tests Made of Spinach in Flats of Sand Sprayed with 2% Uramon Solution and 2% NH<sub>4</sub>NO<sub>3</sub> Solution. Plants Sprayed March 15, 1949.

Date Time	Time	Ura		NO3		
		sprayed	check		sprayed	check
Mar. 16,49	1100	-	-	ti -	-	-
Mar. 18,49	1100		-		-	-

and potassium and tissue tests used to indicate when a deficiency existed. Then, when the deficiency was indicated, the proper element was to be added and tissue tests were to indicate when the deficiency was corrected. For this reason it was necessary that the tests for nutrient element levels indicate a very high reading at the start. The plants were then given a complete solution every other day for six days after which the nitrogen tests indicated a very high nitrogen level.

Applications of selected nutrients were started April 15. Tissue tests made the following day indicated that deficiencies had already developed (Table 17). A nitrogen deficiency developed in the spinach and a phosphorous deficiency developed in the collards when they were given a solution lacking nitrogen, phosphorous, and potassium. Because spinach has a high nitrogen requirement and collards a high phosphorous requirement, the tests indicated these elements as the first limiting factors, as was expected.

When phosphorous alone was applied, no deficiency was indicated in the collards but a nitrogen deficiency was again indicated in the spinach. In some cases it took another two days for the expected deficiencies to develop. In two cases the deficiencies were not indicated by the tests. This could have been due to an accumulation of the element in the flat or possible contamination.

The plan called for correcting deficiencies in one half of the flat and allowing the other half to continue deficient growth until the plant showed visible deficiency signs. It was not possible to correct half of the flat without contaminating the other half so no results were obtained.

The deficiencies indicated by the test were corrected by adding the deficient element to the nutrient solution and applying it to the plants. Tissue tests for the deficient elements were made daily, and after the second day the tests indicated that all deficiencies had been corrected (Table 18).

Date	Nutrients	C. C. Lawrence	Spinach			Collards		
	Fed	N	P	K	N	P	K	
Apr. 16,49	NPK	AA	VH	VH	VH	VH	VH	
	MINUS	L	VH	VH	VH	L	VH	
	P	VH	VH	VH	VH	M	VH	
	K	VH	VH	VH	VH	M	VH	
	N	VH	VH	VH	VH	L	VH	
	PK	VH	VH	VH	VH	VH	VH	
	N P	VH	VH	н	VH	VH	H	
	n k	VH	H	VH	VH	M	VH	
Apr. 18,49	NPK	VH	VH	VH	VH	H	VH	
	MINUS	L	VH	₩	VH	L	VH	
	P	М	VH	VH	VH	VH	VH	
	K	VH	VH	VH	VH	М	VH	
	N	VH	₩	VH	VH	-	VH	
	PK	<b>V</b> H	VH	₩	₩	VH	VH	
	N P	VH	VH	VH	VH	VH	VH	
	NI	TH	н	VH	VH	L	VH	

Table 17. Tissue Tests of Spinach and Collards Indicating the Lowering Levels of Nutrient Elements Within the Plant When Selected Nutrient Solutions were Fed. Feeding of Selected Nutrient Solutions was Started April 15, 1949. Table 18. Tissue Tests of Spinach and Collards Indicating the Correction of Deficient Nutrient Elements in the Plants when the Deficient Element was Added to the Nutrient Solution (added April 18,49). Tests were made until Deficiencies were Indicated Corrected or a Substantial Increase was Indicated.

Date	Nutrients	and the second	Spinac	h		Collar	
	Fed	N	P	K	N	P	K
Apr. 19,49	NPK						
	minus	VL	VH	VH	VH	H	VH
	P	VH	VH	VH	VH	VH	VH
	K	VH				VH	
	N					VH	
	PK	H			VH		
	N P			VН			VH
	NK		VH		1	H	
lpr. 20,49	N P K						
	minus	H			Les Terr	VH	
	Р	a stand					
	K						
	N				Exercise.		
	PK	VH			1		
	N P	a gara			6 ani		
	N K				e sa a		

The results indicate that these tests can be used successfully on spinach and collards, and possibly other members of the cabbage family.

### 6. Miscellanea.

A brief summary of other work and observations related to the subject, but not large enough to be worthy of a section.

## A. Chinese sweet potato (Ipomea Aquatica).

The plants had been grown in a window box arrangement five feet long with a constant level sub-irrigation method of watering. The growing medium was gravel and the plants had been growing quite some time. They had poor color and were evidently pot-bound. A test was made January 8, 1949 and results indicated a nitrogen deficiency. A recheck January 12 and January 27 again indicated a nitrogen deficiency. An application of Hoagland and Arnon (11) solution #1 was made to see if the condition could be corrected. A tissue test on February 15 showed a high nitrogen level in the plant and a short time later the plant had regained its normal green color and growth had increased.

# B. Ornamental asparagus.

The plants were not growing as well as they should due to poor growing weather or lack of nutrients. The new growth of this plant matures and becomes woody rather quickly, making it necessary to test only the newest growth which is very succulent. The tissue test of the new growth indicated a lack of nitrogen while a test of the tuberous root showed a very high nitrogen supply present. Ammonium nitrate was added at the rate of 300 pounds per acre on February 8, 1949. Tests for nitrogen on February 22 and March 4 indicated no nitrogen in either the young or old stems and leaves. On March 12 the tender new growth was tested and a very high nitrogen reaction appeared but faded quickly. Tests made March 15 and 18 indicated no nitrogen present. The question arises here as to the correct method of sampling this plant. It is evident that more work is necessary.

C. A practical use of the test.

A sweet potato plant grown in the greenhouse in sand with nutrient solution showed visual symptoms of what appeared to be a potassium deficiency. Several leaves were yellow and they were dying along the margins. Tissue tests indicated very high reserves of potassium but low nitrogen. A subsequent application of nitrogen corrected the visual symptoms incorrectly diagnosed as a potassium deficiency.

D. A comparison of the results of soil tests made with the Purdue test kit, Simplex test kit, and soils laboratory analyses of a series of field soils.

In an effort to check the results of the Purdue kit's soil tests, comparisons were made with the Simplex soil test kit. A group of soil samples were taken at the Vegetable Research Station at Bixby. The results of the soil tests of these two kits did not check very accurately. The Simplex tests as made, gave results which indicated much lower levels of phosphorous and potassium than the Purdue kit. Reference to Spurway's tables (18) showed that the tables and color charts accompanying the Simplex kit were intended for use with greenhouse soils. Field soils generally have lower concentrations of mineral elements than greenhouse soils. This correlated the tests much better although there was still some difference (Table 19).

To check the tests further, the samples of soil were sent to the soil laboratory for analyses in an effort to find a basis of comparison. A survey of the results indicated there was no basis for comparison. Evidently Spurway (18) has one set of values for nutrient element requirements

Table 19. Results of a Comparison of Soil Tests Made on a Number of Soil Samples with the Purdue Test Kit and Simplex Test Kit Against an Analysis by the Soils Laboratory. The Taylor Test was used in Testing the pH of the Samples in Conjunction with the Simplex Test Kit.

Testing	Test	Sample							
		1	2	3	4	5	6		
	pH P	6.6	6.6	6.6	6.4	6.6	6.6		
Purdue	P	VH	VH	VH	VH	VH	VH		
	K	VH	H	VH	VH	AA	VH		
Taylor	pH	6.6	6.6	6.6	6.6	6.6	6.4		
Simplex*	P	M(1)**	M(글)	H(1)	H(1)	H(1)	H(1)		
	K	$\mathbb{M}(2\overline{2})$	$M(2\frac{1}{2})$	H(5)	M(21)	H(5)	H(5)		
	pH	5.5 to	6.1	7.0	7.0	5.5	to 6.1		
Soils Lab.	P	VH(20)	VH(20)	VH(20)	VH(20)	VH(20)	VH(20)		
Sec. Sec.	K	VL(45)	L(68)	M(106)	M(126)	M(102)	M(115)		

\*The letter readings have been corrected according to Spurway's tables (18) for field soils.

\*\*Figures in parentheses indicate the readings obtained in parts per million.

in parts per million while the soils department has set up another set of values for the same thing. For example, Spurway indicates 20 parts per million of potassium as high while the soils laboratory uses a value of 150-200 parts per million as high. Then again when the letter values of high, low, and medium were compared, the highest value given in the soils laboratory analyses was the lowest in the Simplex tests and the Purdue kit's tests all indicated very high.

The soils laboratory analysis for potassium was made with the "Flame photometer", claimed to be very accurate. The controversy centers over the cobalti-nitrite test for potassium. Test kit results seem to indicate that the test can be used as an index to potassium levels in the soil. At best the quick tests are semi-quantitative and not intended to draw a fine line of difference.

The question arises whether or not the phosphorous and potassium values are too high in the Purdue kit. It has been shown that the soil tests correlate with the tissue tests and they in turn are correlated with the nutrient solutions applied to the plants. The Simplex kit's soil tests are set up for a standard of values arbitrarily decided upon as proper for plant growth.

The pH values of the Purdue kit and Taylor test<sup>3</sup>do not check with those of the soils laboratory. All three tests used indicators. The Purdue test uses brom-thymol blue, the Taylor test benzo red, and the soils laboratory bromcrescl purple. The difference in pH is difficult to explain. The

<sup>&</sup>lt;sup>3</sup> Taylor test - an indicator method for determining the pH of solutions. It uses transmitted light in a comparator block for reading the pH. It is not a part of the Simplex soil test kit.

Purdue and Taylor pH tests were carefully made and, because they check, should be acceptable. The pH test was made in the soils laboratory by an undergraduate student and it is possible for error to be encountered through daily routine analyses. Also their results vary from moderately acid to neutral in the same soil series.

#### SUMMARY AND CONCLUSIONS

1. The purdue tissue test kit indicates the nutrient element levels of nitrogen, phosphorous, and potassium in certain plants. Because the test kit is an indicator and not a "cure all", it is necessary to follow the trends in each crop by testing at regular intervals.

2. Each species of plant should be thoroughly tested before the accuracy of the test kit on those plants is accepted.

3. The tissue test kit was found to indicate nutrient element levels in tomato plants, collards, and spinach. In doing so, it is possible to detect an impending deficiency before it is visible in the plant and to prevent a setback to the crop.

4. The tissue test cannot be used as an infallible guide; all other growth factors must be considered in the diagnostic procedure.

5. It is economically advisable for commercial growers (greenhouse and outdoor) to employ the Purdue soil and tissue test kit on the crops on which it has been proven practical. These are corn, tomatoes, spinach, soybeans, cotton and collards.

6. The tissue test did not indicate nitrogen absorption in spinach when liquid nitrogen sprays were used.

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